



Effects of the roughage/concentrate ratio on the expression of angiogenic growth factors in adipose tissue of fattening *Wagyu* steers

T. Yamada ^{*}, N. Nakanishi

National Institute of Livestock and Grassland Science, Nasushiobara-shi, Tochigi-ken 329-2793, Japan

ARTICLE INFO

Article history:

Received 11 May 2011

Received in revised form 20 July 2011

Accepted 2 November 2011

Keywords:

VEGF

FGF-2

Adipocyte

Beef cattle

ABSTRACT

In this experiment, we studied the effects of the dietary roughage/concentrate ratio on the expression of the angiogenic growth factor (VEGF and FGF-2) and the adipogenic transcription factor (C/EBP β , C/EBP α , and PPAR γ) gene in the adipose tissues of *Wagyu* steers. Steers were fed a high-roughage diet (R group, 35% roughage and 65% concentrate on a TDN basis) or a high-concentrate diet (C group, 10% roughage and 90% concentrate) during the entire fattening period (from 10 to 30 months of age) with the same amount of TDN intake between groups. In mesenteric and intermuscular adipocytes, the expression of the angiogenic growth factors was higher in the R group than in the C group. In contrast, the expression of adipogenic transcription factors in the subcutaneous and intramuscular adipocytes was higher in the C group than in the R group. These results indicate that the dietary roughage/concentrate ratio affects the fat depot-specific differences in the angiogenic and adipogenic gene expression pattern.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The differentiation of preadipocytes into mature adipocytes is regulated by a cascade of transcription factors. Two classes of adipogenic transcription factors, the CCAAT/enhancer-binding protein (C/EBP) family (C/EBP β and C/EBP α) and the peroxisome proliferator-activated receptor γ (PPAR γ), play essential roles during adipogenesis in humans and rodents (Gretchen, Sarah, & Ormond, 1998; Rosen & Spiegelman, 2000; Timchenko, Wilde, Nakanishi, Smith, & Darlington, 1996; Tontonoz, Hu, Graves, Budavari, & Spiegelman, 1994). We previously showed that fattening periods (Yamada, Kawakami, & Nakanishi, 2007), breed differences between *Wagyu* and Holstein (Yamada, Kawakami, & Nakanishi, 2009a), and differences in dietary conditions (Yamada, Kawakami, & Nakanishi, 2009b) affect the expression patterns of the adipogenic transcription factors during bovine adipogenesis.

An extensive capillary network is present in adipose tissues (Crandall, Hausman, & Kral, 1997). Previous studies have indicated that anatomical contacts between adipocytes and blood vessels imply reciprocal influences. Indeed, the inhibition of angiogenesis significantly reduces adipose tissue weight (Nishimura et al., 2007; Rupnick et al., 2002). The adipocyte proliferation takes place within vasculated cell clusters (Cho et al., 2007; Nishimura et al., 2007). Moreover, the adipocyte itself secretes many angiogenic growth

factors such as the vascular endothelial growth factor (VEGF) and the fibroblast growth factor-2 (FGF-2) (Gabrielsson et al., 2002; Miyazawa-Hoshimoto et al., 2005). Recently, we showed that bovine adipocytes express angiogenic growth factors (Yamada, Kawakami, & Nakanishi, 2010).

The carcass value of beef cattle is influenced by both the amount and distribution of adipose tissue. The amount of intramuscular fat is particularly important in determining the quality grades of beef. Especially, Japanese black (*Wagyu*) cattle are characterized by the ability to accumulate high amount of intramuscular fat (Zembayashi & Lunt, 1995). The dietary roughage/concentrate ratio has been shown to affect carcass fat deposition. The quality of roughage-finished and grain-finished carcasses has been compared (Bowling, Smith, Carpenter, Dutson, & Oliver, 1977; Muramoto, Aikawa, Shibata, & Nakanishi, 2002; Tatum, Klein, Williams, & Bowling, 1988); however, a problem with such a comparison is that the total digestible nutrient (TDN) intake of the high-roughage diet group is lower than that of the high-concentrate diet group. If cattle from the two management systems are slaughtered on a time-constant basis, these results are synonymous with a comparison of “lean” and “obese” animals. Therefore, in the present study, we investigate the adipogenesis of *Wagyu* steers fed a high-roughage or a high-concentrate diet during the entire fattening period (from 10 to 30 months of age) with the same amount of TDN intake between groups. On the other hand, the effects of the dietary conditions on the expression of the angiogenic growth factors in adipocytes, including humans and rodents, are still unclear. The main objective of the present study was to elucidate the effects of the dietary roughage/concentrate ratio during the entire fattening period on the expression of the angiogenic (VEGF and FGF-2) and adipogenic (C/EBP β , C/EBP α ,

^{*} Corresponding author at: National Institute of Livestock and Grassland Science, 768 Senbonmatsu, Nasushiobara-shi, Tochigi-ken 329-2793, Japan. Tel.: +81 287 37 7811; fax: +81 287 36 6629.

E-mail address: toyamada@affrc.go.jp (T. Yamada).

and PPAR γ) genes in the adipose tissues of *Wagyu* steers from various anatomical sites (subcutaneous, mesenteric, intermuscular, and intramuscular).

2. Materials and methods

2.1. Animals

Eight *Wagyu* steers were used in this study. They received concentrate (88% total digestible nutrients (TDN) and 15% crude protein (CP)) and orchard grass hay (56% TDN and 8% CP) until they were slaughtered at 30 months of age as described below. At 10 months of age, the steers were allotted by body weight to two groups, (1) the high-roughage diet group (R group, $n=4$) and (2) the high-concentrate diet group (C group, $n=4$). The R group was fed a high-roughage diet consisting of 35% roughage and 65% concentrate, and the C group was fed a high-concentrate diet consisting of 10% roughage and 90% concentrate (on a TDN basis). In the present study, the steers were pair-fed in order to eliminate the influence of the total TDN intake between groups. At 30 months of age, adipose tissue samples from four types of adipose tissues (subcutaneous, mesenteric, intermuscular, and intramuscular) were collected at slaughter. The left side of the carcass was sectioned between the 3rd and 4th lumbar vertebrae. The subcutaneous and intermuscular adipose tissues surrounding the longissimus muscle and the intramuscular adipose tissue within the longissimus muscle were collected from the section. The mesenteric fat was sampled from the area surrounding the colon. All adipose tissue samples were collected immediately after slaughter. Collected samples were stored at -80°C in RNA-later reagent (Ambion, CA, USA) until RNA extraction. After slaughter, the left side of the carcass was chilled for 72 h at 4°C and physically separated into muscle, bone, and fat to measure the fat tissue weight. The intramuscular fat content of the longissimus muscle was obtained via Soxhlet extraction method. All animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals (National Institute of Livestock and Grassland Science).

2.2. RNA isolation and real-time PCR

Angiogenic and adipogenic gene expressions were analyzed by real-time PCR as described previously (Yamada et al., 2010). In brief, total RNA was extracted from adipose tissue using the RiboPure kit (Ambion) according to the manufacturer's instructions. The first-strand cDNA was reverse-transcribed from 0.5 μg total RNA using the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) according to the manufacturer's protocol. Real-time PCR was performed with a Mini Opticon (Bio-Rad, Munich, Germany) using SYBR Green Real-time PCR Master Mix-Plus (Toyobo) according to the manufacturer's instructions. The primer sequences were as follows: VEGF, 5'-GAA CTT TCT GCT CTC TTG GG-3' (forward) and 5'-CTG GCT TTG GTG AGG TTT GA-3' (reverse); FGF-2, 5'-ACC GGT CAA GGA AAT ACT CCA G-3' (forward) and 5'-CAG GTC CTG TTT TGG GTC CA-3' (reverse); C/EBP β , 5'-ACA GCG ACG AGT ACA AGA TCC-3' (forward) and 5'-GAC AGT TGC TCC ACC TTC TTC T-3' (reverse); C/EBP α , 5'-CCG TGG ACA AGA ACA AGC AAC-3' (forward) and 5'-TGG TCA GCT CCA GCA CCT TC-3' (reverse); PPAR γ , 5'-GTG AAG TTC AAC GCA CTG GA-3' (forward) and 5'-ATG TCC TCA ATG GGC TTC AC-3' (reverse); ribosomal protein large P0 (RPLP0), 5'-CAA CCC TGA AGT GCT TGA CAT-3' (forward) and 5'-AGG CAG ATG GAT CAG CCA-3' (reverse). The reaction conditions were designed as follows: initial denaturation at 95°C for 60 s followed by 40 cycles at 95°C for 15 s, 55°C for 15 s, and 70°C for 30 s. SYBR green fluorescence was detected at the end of each cycle to monitor the amount of real-time PCR product formed during that cycle. The specificity of the PCR products was determined by melting curve analysis at the end

of each run. The standard curve of each product followed the calculation of respective gene expressions. The expression levels of adipogenic and angiogenic mRNA were normalized by RPLP0 as an internal control (Ohsaki et al., 2007; Yamada et al., 2010).

2.3. Adipocyte cellularity

The samples of adipose tissue (subcutaneous, mesenteric, intermuscular, and intramuscular) were fixed with osmium tetroxide according to the method of Robelin (1981). Slices of adipose tissue were rinsed in 0.154 M NaCl and then fixed with 50 mM collidine-HCl buffer (pH 7.4) containing 2% osmium tetroxide. The slices were left in the fixative for 96 h at room temperature. After fixation, the samples were rinsed in 0.154 M NaCl for 24 h at room temperature. The fixed adipocytes were then isolated in a urea solution as reported by Etherton, Thompson, and Allen (1977). Fixed adipose tissue samples were placed into 8 M urea in 0.154 M NaCl for 48 h at room temperature. Fixed and urea-isolated adipocytes were separated into 0.01% Triton X-100 in a 0.154 M NaCl buffer (pH 10). The adipocyte diameter was measured using WinRoof software (Mitani Corporation, Fukui, Japan). Over 300 adipocytes for each sample were measured according to the method of Lavau, Susini, Knittle, Blanchet-Hirst, and Greenwood (1977).

2.4. Statistical analysis

All results are presented as the means $\pm$ S.D. The differences in mean values between the groups were analyzed by Student's *t*-test using the StatView Version 5 program (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Growth performance and carcass data of *Wagyu* steers

Table 1 shows the effects of the dietary roughage/concentrate ratio on the growth performance and feed intake of *Wagyu* steers. There were no differences in the body weight and daily gain between groups. The feed intakes of steers during fattening periods were virtually identical to the amounts specified in the experiment design. The TDN intake from the roughage was significantly higher in *Wagyu* steers fed a high-roughage diet than in those fed a high-concentrate diet. However, there were no significant differences in the total (roughage plus concentrate) TDN intakes between groups. Table 2 shows the effects of the dietary roughage levels on the carcass traits and regional variation of fat deposition in *Wagyu* steers. There were

Table 1
Growth performance and feed intake of *Wagyu* steers.

	R	C
<i>Body weight (kg)</i>		
Initial weight (10 months)	302.5 $\pm$ 26.3	298.5 $\pm$ 25.3
Final weight (30 months)	648.2 $\pm$ 28.5	667.0 $\pm$ 26.4
<i>Daily gain (kg/day)</i>		
(from 10 to 30 months)	0.67 $\pm$ 0.06	0.68 $\pm$ 0.01
<i>Feed intake during fattening period (kg/day)</i>		
Roughage	3.60 $\pm$ 0.20a	1.25 $\pm$ 0.02b
Concentrate	4.93 $\pm$ 0.09a	6.66 $\pm$ 0.14b
<i>TDN intake during fattening period (kg/day)</i>		
From roughage	1.77 $\pm$ 0.09a	0.61 $\pm$ 0.01b
From concentrate	3.78 $\pm$ 0.07a	5.10 $\pm$ 0.10b
Roughage plus concentrate	5.56 $\pm$ 0.10	5.71 $\pm$ 0.11

Values are expressed as Mean $\pm$ S.D.

High-roughage feeding group (R, $n=4$), High-concentrate feeding group (C, $n=4$).

a,b: Values with different superscripts in same line are significantly different ($P<0.05$).

Download English Version:

<https://daneshyari.com/en/article/5792627>

Download Persian Version:

<https://daneshyari.com/article/5792627>

[Daneshyari.com](https://daneshyari.com)